Evaluation of the Sensory, Physical, Chemical Properties and antioxidant activity of Gluten-Free Biscuits Made from Cornflour with the Addition of Stevia leaf powder and *Moringa oleifera* leaf powder

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Abstract:
The demand for gluten-free bakery products has been increasing in recent years. However, research on the use of Stevia leaf powder in combination with *Moringa oleifera* leaf powder in such products remains limited. Therefore, this study aimed to develop and evaluate gluten-free biscuits made from corn flour fortified with *Moringa oleifera* leaf powder and stevia leaf powder as a sugar substitute.
The sensory evaluation results showed that the corn flour biscuits significantly outperformed the conventional wheat flour biscuits (control sample) in most sensory attributes. The fortified biscuits achieved a higher overall acceptability score (9.8 vs. 8.6) and exhibited a more attractive color, receiving a perfect score of 10, which was attributed to the presence of phenolic and flavonoid compounds in *Moringa oleifera* leaf powder and bioactive compounds in stevia. Aroma and after-chew mouthfeel also received perfect scores of 10, while texture scored an excellent 9, likely due to the high dietary fiber content.
In addition to their superior sensory quality, the fortified biscuits demonstrated improved physical properties, including increased thickness and diameter while maintaining a satisfactory spread ratio.
Chemical analysis revealed significant nutritional improvements, including higher levels of protein, dietary fiber, and ash (mineral content), reaching 26.70%, 20.5%, and 11.8%, respectively. These improvements were attributed to the incorporation of stevia and *Moringa oleifera* leaf powders, which are naturally rich in these components. Meanwhile, the total carbohydrate content decreased significantly to 18.1% compared with the conventional biscuits.
The antioxidant activity of *Moringa oleifera* leaf extracts, determined using the DPPH assay, showed that free radical scavenging activity increased with increasing extract concentration, reaching 19.05%, 38.98%, 57.44%, 69.08%, and 73.65% at concentrations of 30, 60, 120, 250, and 500 ppm, respectively. Phytochemical analysis further showed antioxidant-related compounds at levels of 44.25, 98.9, 74.0, and 32.6 mg/g for alkaloids, phenolics, flavonoids, and tannins, respectively.
These findings demonstrate that replacing less healthy ingredients with nutritionally beneficial alternatives represents an innovative strategy for developing healthier food products. Furthermore, combining stevia leaf powder with *Moringa oleifera* leaf powder helps address an existing research gap while enhancing both consumer acceptability and the nutritional value of gluten-free biscuits, making them a healthier and more distinctive alternative to conventional wheat biscuits.
Keywords: Biscuits, Corn flour, Stevia leaf powder, *Moringa oleifera* leaf Powder, Rice bran oil.

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1. Introduction

Gluten-free products have gained considerable popularity in recent years due to increasing consumer preference and prevailing scientific and health trends (2). These products are particularly important for individuals with gluten intolerance or celiac disease, a chronic immune-mediated disorder triggered by gluten that affects approximately 1% of the global population (13). The only effective treatment for this condition is lifelong adherence to a gluten-free diet, which has led to a significant increase in the demand for gluten-free foods (19).

Among bakery products, biscuits are one of the most widely consumed food items because they are readily available in supermarkets and local retail stores (27). Their popularity is attributed to their nutritional value, affordability, ease of accessibility, wide variety of flavors, convenience of consumption, and extended shelf life (19).

Wheat flour is the primary ingredient used in biscuit production because it contains gluten, a functional protein that plays a critical role in determining the quality of baked products. Gluten contributes to the formation of the dough structure and is responsible for its cohesiveness, elasticity, strength, and the texture of the final product (12, 29).

However, unlike bread and several other baked products, gluten is not essential for biscuit production. Consequently, wheat flour can be successfully replaced with gluten-free alternatives without substantially compromising product quality (13). Based on this concept, corn flour, together with stevia leaf powder and moringa leaf powder, has emerged as a promising combination for the development of gluten-free biscuits.

Corn flour is a naturally gluten-free ingredient that is widely available locally and is generally less expensive than wheat flour. In recent years, increasing attention has been directed toward the development of gluten-free bakery products based on corn flour. For example, biscuits formulated with different proportions of corn, rice, and soybean flours have been reported, with flour composition significantly influencing both the nutritional value and sensory quality of the final product (19). Similarly (5), investigated the characteristics of biscuits produced from rice flour and sorghum flour using different ingredient formulations. Furthermore, (28) developed gluten-free biscuits from corn flour fortified with walnuts and peanuts.

Stevia (*Stevia rebaudiana*) leaves, which have recently been introduced into Iraq, are a rich source of naturally occurring sweet diterpene glycosides, including *ent*-kaurene diterpenoid glycosides, particularly rebaudioside A and stevioside, which are the principal compounds responsible for their intense sweetness. Stevia leaf powder can therefore partially or completely replace conventional sugar in food formulations (31,24).

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Image (1): *Stevia rebaudiana* plant

Moringa oleifera, commonly known as the miracle tree, is a fast-growing plant that is exceptionally rich in vitamins, minerals, and antioxidants. Its leaves, roots, and seeds have been widely used in traditional medicine and therapeutic nutrition to enhance immune function, reduce inflammation, regulate blood glucose levels, and support cardiovascular health (38). *Moringa oleifera* contains a wide range of naturally occurring phytochemicals, including phenolic acids, flavonoids, carotenoids, glucosinolates, and tannins, making it a promising ingredient for functional food applications. Among its major bioactive compounds are cryptochlorogenic acid, isoquercetin, and astragalin, which are largely responsible for its antioxidant, antihypertensive, and anti-inflammatory properties (7,40).



Image (2): *Moringa oleifera* (miracle tree): botanical description and its major health-promoting properties

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There is a growing need to develop fortified bakery products, such as biscuits, that are widely accepted and commonly consumed as snack foods to help meet nutritional requirements and improve the health of vulnerable population groups. Biscuits also provide a suitable vehicle for the incorporation of essential micronutrients and protein to address nutritional needs (30). Previous studies have demonstrated the feasibility of replacing wheat flour with corn flour in biscuits and other bakery products. However, limited research has focused on the development of gluten-free biscuits supplemented with stevia leaf powder and moringa leaf powder, particularly with respect to their physical characteristics, nutritional quality, and antioxidant capacity. Therefore, the objectives of the present study were to: (1) develop gluten-free biscuits based on corn flour supplemented with *moringa* leaf powder and *stevia* leaf powder; (2) evaluate their sensory, physical, chemical, and antioxidant properties in comparison with conventional wheat biscuits; and (3) assess the nutritional improvements achieved through this formulation. In addition, this study aims to provide a healthier alternative for individuals with gluten intolerance while contributing to the development of gluten-free bakery products. The developed biscuits will also be used as a dietary component in future experimental animal studies**.**

2. Materials and Methods

The experimental work was conducted at the Directorate of Science and Technology, Baghdad. Biscuits were prepared using different flour substitution formulations to produce products with enhanced nutritional value and improved health benefits, as described below:

2.1. Biscuit Preparation

Control Sample: The control biscuits were prepared using premium wheat flour (72% extraction rate) according to the method described by (22). The ingredients and their respective quantities are presented in Table 1. The dough was mixed for 10 minutes until a smooth and homogeneous consistency was achieved, then refrigerated at 6°C for 30 minutes. Subsequently, the dough was rolled out, cut into circular discs, and baked on an aluminum baking tray in an electric oven at 180°C for 6 minutes**.**

Table (1): Formulation of wheat flour biscuits (control sample)

Ingredient	Percentage (%)
Wheat flour	**100**
Fat	**45**
Powdered sugar	**30**
Egg	**50**
Vanilla	**2**

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Baking powder	**1**

Ingredient Substitution Sample Supplemented with *Moringa Leaf* Powder:

Gluten-free biscuits were prepared using 100% corn flour according to the method described by (22). The ingredients and their respective quantities are presented in Table 2. Selected ingredient substitutions were made, and moringa (*Moringa oleifera*) leaf powder was incorporated into the formulation.

Table (2): Formulation of corn flour biscuits supplemented with *Moringa oleifera* leaf powder

Ingredient	Percentage %
Corn flour	100
Rice bran oil	45
Stevia leaf powder	30
Egg	50
Vanilla	2
Baking powder	1
Moringa oleifera leaf powder	2

2.2. Sensory Evaluation of Corn Flour Biscuits Supplemented with *Moringa oleifera* Leaf Powder:

Table (3): Sensory evaluation score sheet.

Attribute	Evaluation

The dough was mixed for 10 minutes until a smooth and homogeneous consistency was obtained, then refrigerated at 6°C for 30 minutes. It was subsequently shaped using biscuit molds and baked on an aluminum baking tray in an electric oven at 180°C for 6 minutes. After baking, the biscuits were allowed to cool at room temperature for 30 minutes before being packaged in polyethylene bags and stored until further analyses.

Sensory evaluation was conducted at the Department of Food Science, College of Agriculture, Tikrit University. The sensory panel consisted of ten trained panelists who were faculty members specializing in food science and sensory analysis. The panelists ranged in age from 45 to 60 years and included four females and six males, all of whom possessed extensive experience in sensory evaluation.

Consumer acceptability and preference were assessed based on the following sensory attributes: appearance, texture, taste, aroma, color, overall acceptability, and mouthfeel after chewing, as shown in Table 3. A ten-point hedonic scale was used for the evaluation, with scores ranging from 1 to 10, where 10 indicated "like extremely" and 1 indicated "dislike extremely" (4).

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Attribute	Evaluation
Appearance	Like extremely ← Dislike extremely
Texture	Like extremely ← Dislike extremely
Flavor	Like extremely ← Dislike extremely
Aroma	Like extremely ← Dislike extremely
Color	Like extremely ← Dislike extremely
Overall acceptability	Like extremely ← Dislike extremely
Mouthfeel after chewing	Like extremely ← Dislike extremely

2.3. Physical Properties of Corn Flour Biscuits Supplemented with Moringa oleifera Leaf Powder:

Biscuit diameter (W, cm): The diameter was determined by placing six biscuit samples edge to edge in a straight line and measuring their total diameter. The biscuits were then rotated by 90°, and a second measurement was taken. The average of the two measurements was recorded as the biscuit diameter (6).

Biscuit thickness (T, cm): Six biscuit samples were stacked vertically, and their total height was measured. The average thickness was then calculated and recorded (6).

Spread ratio: The spread ratio was calculated by dividing the biscuit diameter (W) by its thickness (T), according to Equation (1):
Spread Ratio= W/T

Equation (1). Calculation of the spread ratio.

2.4 Chemical Analysis of Corn Flour Biscuits Supplemented with *Moringa oleifera* Leaf Powder

Moisture Content
Moisture content was determined as the percentage weight loss of the sample after oven drying, following the (8) method. Approximately 3 g of each sample was accurately weighed into a pre-weighed moisture dish and dried in a hot-air oven at 105°C for 8 h. After drying, the moisture content was calculated according to Equation (2):
Moisture (%)= Weight before Drying - Weight after drying / Weight before drying×100

Equation (2). Calculation of moisture content.

Crude Protein
Crude protein content was determined using the Kjeldahl method according to (39).
Approximately 5 g of the sample was weighed into a Kjeldahl digestion flask, followed by the addition of 25 mL of concentrated sulfuric acid and an appropriate amount of a catalyst mixture consisting of potassium sulfate and copper sulfate. The mixture was digested by heating until a clear pale-blue solution was obtained. After digestion, the digest was quantitatively transferred to a Kjeldahl distillation unit containing 40% sodium hydroxide solution. The liberated ammonia was distilled into a receiving flask containing a known volume of 20% boric acid with methyl red and bromocresol blue as mixed

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indicators. Distillation was continued until approximately 25 mL of distillate had been collected. The collected distillate was then titrated with 0.1 N hydrochloric acid (HCl). A reagent blank was prepared using the same chemicals without the sample. Crude protein content was calculated according to Equation (3):

Protein (%)=Volume of HCl consumed× Normality×0.014×6.25/ Sample weight×100

Equation (3). Calculation of crude protein content.

Ash Content

Ash content was determined according to the(8) method. The sample was placed in a pre-weighed porcelain crucible and incinerated in a muffle furnace at 525°C for 3 h. After cooling, the ash content was calculated using Equation (4):

Ash (%) = (Weight of crucible + ash) - Weight of empty crucible / Sample weight×100

Equation (4). Calculation of ash content.

Crude Fat

Crude fat content was determined according to the (8) method using a Soxhlet extraction apparatus. Approximately 10 g of the dried sample was weighed, wrapped in filter paper, and placed in a Soxhlet extraction thimble. A clean, dry extraction flask was weighed before use, after which 250 mL of hexane was added as the extraction solvent. The extraction process was continued for approximately 5 h. Upon completion, the solvent was recovered, and the extraction flask was removed and placed in a hot-air oven at 80°C for 30 min to ensure complete evaporation of any residual hexane, leaving only the extracted fat. The flask was then cooled to room temperature and weighed. The crude fat content was calculated according to Equation (5):

Crude Fat (%)=Weight of flask after extr action - Weight of empty flask / Sample weight× 100

Equation (5). Calculation of crude fat content.

Crude Fiber

Crude fiber was determined according to the method described by (26). Crude fiber is defined as the portion of the food material that is insoluble after sequential digestion with 1.25% sulfuric acid and 1.25% sodium hydroxide, each applied under boiling conditions for 30 min. It consists primarily of cellulose, hemicellulose, and lignin.

Following fat extraction, the sample was sequentially digested with 1.25% sulfuric acid and 1.25% sodium hydroxide. The insoluble residue was then filtered and consisted of crude fiber, insoluble ash, and residual moisture. The residue was subsequently dried, weighed, incinerated, and reweighed. The loss in weight after incineration was considered the crude fiber content (26).

The crude fiber content was calculated according to Equation (6):

Crude Fiber (%) = Weight of crude fiber / Dry sample weight×100

Equation (6). Calculation of crude fiber content.

2.5. Antioxidant-Related Phytochemical Analysis of *Moringa oleifera* Leaf Powder

Total Alkaloid Content

The presence of alkaloids was initially confirmed using Dragendorff's test. A

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portion of the extract was dissolved in dilute hydrochloric acid (HCl), and two drops of Dragendorff's reagent were added. The formation of an orange crystalline precipitate indicated the presence of alkaloids, after which the sample was subjected to quantitative analysis.

For alkaloid determination, a portion of the dried extract residue was dissolved in 2 N hydrochloric acid (HCl) and filtered. One milliliter of the filtrate was transferred to a separatory funnel and washed with 10 mL of chloroform. The pH of the solution was adjusted using 0.1 N sodium hydroxide (NaOH), followed by the addition of 5 mL of bromocresol green (BCG) solution and 5 mL of phosphate buffer. Separately, 20 g of the sample was extracted with methanol for 24 h using a Soxhlet extraction apparatus. The extract was filtered, and the methanol was removed under reduced pressure using a rotary evaporator at 45°C until complete dryness. The total alkaloid content was then determined according to the method described by (37).

Standard Calibration Curve

Accurately measured aliquots (0.4, 0.6, 0.8, and 1.0 mL) of a standard atropine solution were transferred into separate separatory funnels. Subsequently, 5 mL of phosphate buffer (pH 4.7) and 5 mL of bromocresol green (BCG) solution were added. The mixture was extracted successively with increasing volumes of chloroform (1, 2, 3, and 4 mL). The chloroform extracts were combined in a 10 mL volumetric flask, and the volume was adjusted to the mark with chloroform.

The absorbance of the resulting chloroform phase was measured at 470 nm using a Shimadzu UV-1800 UV–Visible spectrophotometer, against a reagent blank prepared under identical conditions without the addition of the atropine standard solution (3).

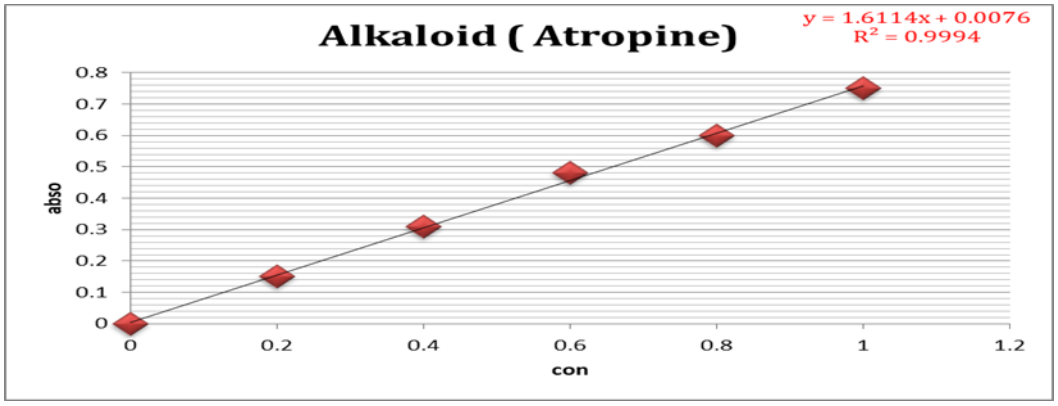


Figure (1): Standard calibration curve of atropine determined by UV–Visible spectrophotometry

Total Phenolic Content (TPC)

The total phenolic content (TPC) of the ethanolic extract was determined using the Folin–Ciocalteu reagent. The reaction mixture consisted of 100 µL of the extract, 500 µL of Folin–Ciocalteu reagent, and 1.5 mL of 20% sodium carbonate ($Na_2 CO_3$) solution. The mixture was thoroughly mixed using a

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vortex mixer and diluted to a final volume of 10 mL with distilled water. After incubation for 2 h at room temperature, the absorbance was measured at 765 nm using a UV–Visible spectrophotometer. The total phenolic content was quantified from a calibration curve prepared with gallic acid as the standard and expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW) (23).

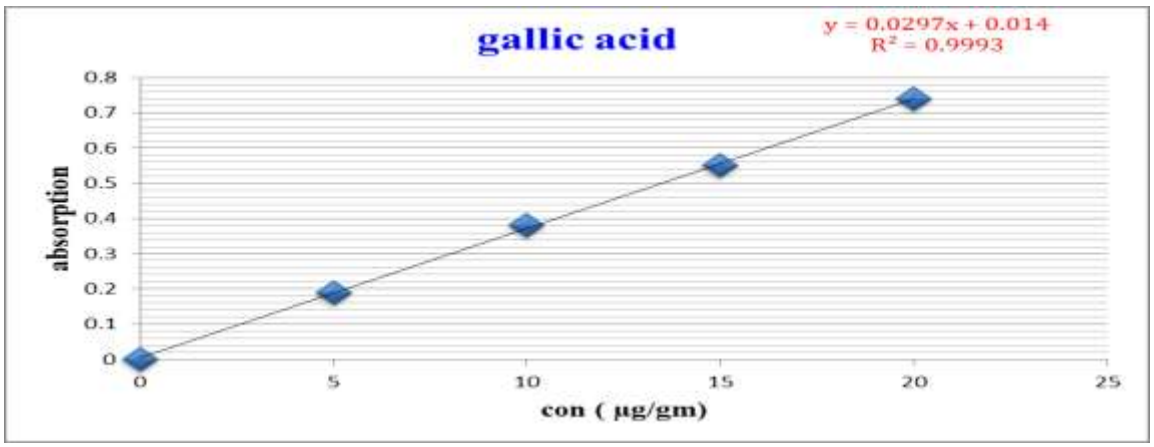


Figure (2): Gallic acid standard calibration curve for the determination of total phenolic content

Total Flavonoid Content (TFC) The total flavonoid content (TFC) of the crude extract was determined using the aluminum chloride colorimetric method. Briefly, 50 µL of the crude extract (1 mg/mL in ethanol) was diluted to 1 mL with methanol. The diluted extract was then mixed with 4 mL of distilled water and 0.3 mL of 5% sodium nitrite ($NaNO_2$) solution. After incubation for 5 min, 0.3 mL of 10% aluminum chloride ($AlCl_3$) solution was added, and the mixture was allowed to stand for 6 min. Subsequently, 2 mL of 1 M sodium hydroxide (NaOH) solution was added, and the final volume was adjusted to 10 mL with distilled water. The reaction mixture was allowed to stand for 15 min, after which the absorbance was measured at 510 nm using a UV–Visible spectrophotometer. The total flavonoid content was calculated from a calibration curve prepared using rutin as the reference standard and expressed as milligrams of rutin equivalents per gram of dry weight (mg RE/g DW) (16).

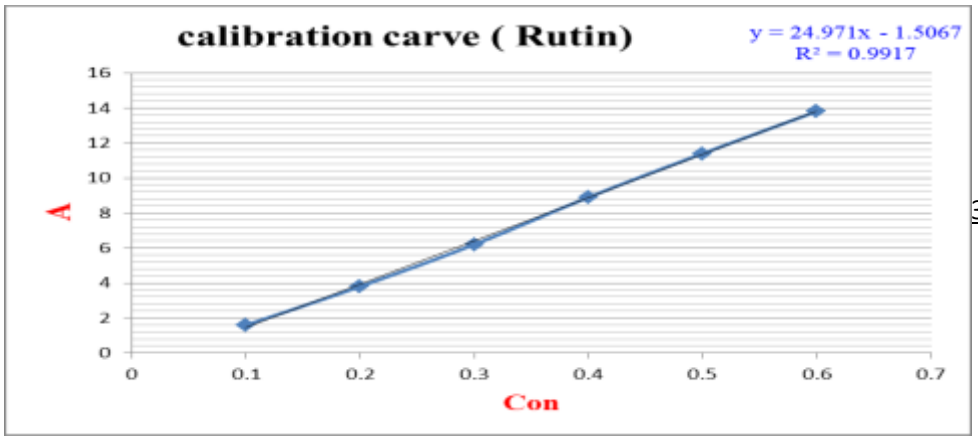


Figure (3): Rutin standard calibration curve used for the determination of total flavonoid content.

Total Tannin Content

The total tannin content was determined according to the method described by Abdelkader et al. (2014). Briefly, 2 g of the sample extract was mixed with a water–ethanol solution (20:80, v/v) and heated in a water bath. The mixture was then filtered, and a few drops of ferric chloride ($FeCl_3$) solution were added to the filtrate. The development of a dark green color indicated the presence of tannins.

For further confirmation, 1 mL of the extract was mixed with 2 mL of 2% sodium chloride (NaCl) solution. The mixture was filtered and then combined with 5 mL of 1% gelatin solution. The formation of a precipitate confirmed the presence of tannins. The absorbance of the reaction mixture was subsequently measured at 540 nm using a UV–Visible spectrophotometer (1).

3. Results and Discussion

3.1. Sensory Evaluation

The sensory evaluation results of the corn flour biscuits supplemented with *Moringa oleifera* leaf powder, in comparison with the control sample, are presented in Table (4).

Table (4): Sensory evaluation results of corn flour biscuits supplemented with *Moringa oleifera* leaf powder compared with the control sample

Sensory Attribute	Studied Sample	Control Sample
Appearance	9.5	8.5
Texture	9.0	7.5
Flavor	8.8	8.5
Aroma	10.0	9.0
Color	10.0	8.5
Overall acceptability	9.8	8.6
Mouthfeel after chewing	10.0	9.0

The sensory evaluation was conducted using a 10-point hedonic scale, where a score of 10 indicated (like extremely), while a score of 1 indicated (dislike extremely). The overall mean scores obtained for the studied biscuit samples ranged from (8.8 to 10).

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The sensory evaluation results presented in **Table (4)** revealed a noticeable difference between the control sample (wheat flour biscuits) and the studied sample (corn flour biscuits fortified with stevia leaf powder and moringa leaf powder). The evaluation conducted by **10 trained panelists** indicated a preference for the fortified biscuits in most sensory attributes. The studied sample achieved a higher overall acceptability score (**9.8**) compared with the control sample (**8.6**), demonstrating the successful replacement of conventional ingredients with health-promoting components to produce biscuits with greater acceptability and consumer preference.

The studied sample achieved the maximum score (**10**) for aroma, color, and mouthfeel after chewing, outperforming the control sample, which received scores of **9, 8.5, and 9**, respectively. This improvement in sensory characteristics may be attributed to the presence of natural compounds in the added stevia and moringa powders, which contributed to the development of an attractive golden color and enhanced sensory appeal (36).

The improved texture may be related to the combined content of dietary fiber, protein, minerals, and antioxidants present in corn flour, stevia leaf powder, and moringa leaf powder. This formulation may contribute to enhanced moisture retention and provide a more homogeneous internal structure (crumb) with improved palatability during chewing (20).

3.2. Physical Tests:

The physical measurements showed that the studied sample had a larger average diameter (28 cm compared with 24 cm) and a lower spread ratio (1.86 compared with 1.77) as presented in Table (5). The spread ratio is considered a critical indicator of bakery product quality, as it depends on the balance between dough viscosity and the expansion forces generated by water vapor and fermentation gases during baking (21).

The main factor responsible for this variation was the complete replacement of wheat flour with corn flour. Gluten proteins in wheat flour form an elastic network that provides dough cohesiveness and the ability to retain gases, thereby limiting spread during baking. In contrast, the absence of this gluten network in gluten-free corn flour generally results in reduced viscosity and increased spread during the baking process (26).

However, the results indicated a lower spread ratio for the studied sample despite the absence of gluten in corn flour. This may be attributed to the compensatory effect of the added dietary fibers present in stevia and moringa leaf powders. These components contributed to obtaining a larger diameter compared with the control sample while maintaining a lower spread ratio.

These findings are consistent with those reported by (10), who found that the incorporation of fruit and vegetable powders increases the hardness of baked products and reduces their spread, particularly in gluten-free formulations, due to enhanced water absorption and improved dough stability.

Table (5): Physical test results of corn flour biscuits supplemented with stevia leaf powder and moringa leaf powder compared with the control sample..

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Physical Parameter	Studied Sample	Control Sample
Diameter (cm)	28.0	24.0
Thickness (cm)	15.0	13.5
Spread ratio	1.86	1.77

The results presented in Table (5) also showed a noticeable increase in biscuit thickness for the studied sample (15 cm) compared with the control sample (13.5 cm). This increase can be directly attributed to changes in the formulation. The dietary fibers present in stevia and moringa leaf powders not only enhance water binding but also contribute to the formation of a more compact and stable structure during baking. In addition, these fibers, together with other bioactive constituents, influence the texture of the biscuits, while the naturally occurring sugars may participate in caramelization reactions, thereby stabilizing the product structure at an early stage of baking. Consequently, the absence of the gluten network was compensated for by the water-binding and water-retention capacity of the dietary fibers, resulting in a thicker final product (43).

3.3. Chemical Analysis

Table (6): Chemical analysis results of corn flour biscuits supplemented with stevia leaf powder and moringa leaf powder compared with the control sample

Chemical Parameter	Concentration (%)
Moisture	23.55
Protein	26.70
Ash	11.80
Fat	10.00
Crude fiber	20.50
Total carbohydrates	7.45

The results presented in Table (6) showed a markedly high protein content (26.7%), despite the fact that corn flour generally contains less protein than wheat flour. This increase can be attributed to the combined incorporation of stevia and moringa leaf powders, both of which are valuable sources of plant-based protein, containing approximately 10–20% protein on a dry weight basis (14). In addition, moringa leaf powder contains moderate amounts of protein, and its contribution to the overall protein content increases with higher levels of incorporation into the biscuit formulation (32).

The elevated protein content enhances the nutritional value of the product and may promote satiety, making these

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biscuits a healthier dietary option that can contribute to meeting daily protein requirements as part of a balanced diet.
The crude fat content of the formulated biscuits was approximately 10%, reflecting both the naturally occurring lipids in corn flour and the added fat source used during processing. Healthy oils, such as rice bran oil, are commonly incorporated into biscuit formulations because of their high content of unsaturated fatty acids and antioxidant compounds. Furthermore, moringa leaf powder contains phenolic compounds and flavonoids with well-documented antioxidant activity, making it a functional ingredient capable of further enhancing the nutritional quality of the product (42). The fat content can therefore be maintained within recommended nutritional limits through the use of healthy vegetable oils, particularly rice bran oil.
The relatively high ash content (11.8%) shown in Table (6) indicates that the formulated biscuits are rich in minerals. This finding is attributed to the use of whole corn flour together with stevia and moringa leaf powders, all of which are excellent sources of essential minerals, including potassium, magnesium, calcium, iron, and zinc (24). Compared with conventional white biscuits, which typically contain less than 2% ash, the substantially higher ash content provides strong evidence that incorporating these mineral-rich natural ingredients successfully enhanced the nutritional value of the product.
The moisture content (23.55%) may be explained by the high water-holding capacity of the added ingredients, particularly the dietary fibers present in stevia and moringa leaf powders, which are capable of retaining considerable amounts of water within the biscuit matrix (35).
The crude fiber content (20.5%) represents another important finding and is consistent with the observations reported by (18). This high fiber level reflects the use of whole corn flour together with stevia and moringa leaf powders. Although such a high fiber content may influence shelf life and therefore requires appropriate packaging and storage conditions, it also contributes to a softer, moister texture in the final product, which is considered a desirable quality attribute in many biscuit types. Moreover, this level of fiber places the product within the high-fiber category according to commonly accepted nutritional standards. High dietary fiber intake has been associated with improved digestive health, better glycemic control, and enhanced satiety.
The results also demonstrated a substantial reduction in total carbohydrate content (7.45%) compared with conventional biscuits, which may contain more than 70% carbohydrates. This reduction is primarily attributable to the modified formulation, particularly the use of stevia leaf powder as the principal sweetening ingredient in place of sucrose (table sugar). Consequently, the carbohydrate and caloric contents were markedly reduced, producing a low-carbohydrate biscuit that may be particularly suitable for diabetic dietary regimens and may contribute to improved blood glucose management without compromising sweetness. Nevertheless, these potential health benefits should be confirmed through glycemic index determination and well-designed clinical studies (10).

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3.4. Antioxidant Analysis of *Moringa oleifera* Leaf Powder

The results of the antioxidant-related analyses performed on *Moringa oleifera* leaf powder are presented in Table (7).

Table (7): Results of the antioxidant-related analyses of *Moringa oleifera* leaf powder

Test	Concentration (mg/g)
Total alkaloid content	44.25
Total phenolic content	98.90
Total flavonoid content	74.00
Total tannin content	32.60

The results demonstrated that *Moringa oleifera* leaves contain high concentrations of bioactive phytochemicals, including alkaloids, phenolic compounds, flavonoids, and tannins. This finding reflects the rich phytochemical composition of moringa leaves, as these compounds are well recognized for their antioxidant, anti-inflammatory, and other pharmacological properties.

As shown in Table (7), the total alkaloid content was 44.25 mg/g, which was lower than the levels of total phenolics and flavonoids. Nevertheless, alkaloids are considered pharmacologically important because of their diverse biological activities, including antimicrobial and anti-inflammatory effects. Variations in alkaloid content may be attributed to the extraction solvent employed, since different alkaloids exhibit varying solubilities in acidic media and organic solvents compared with water (11).

The results also revealed a high total phenolic content (98.9 mg/g), which may be attributed to the ability of *Moringa oleifera* leaves to synthesize a wide range of phenolic compounds, including gallic acid, caffeic acid, and chlorogenic acid. High phenolic content is considered an important indicator of antioxidant capacity because these compounds can donate electrons or hydrogen atoms to neutralize free radicals and thereby inhibit oxidative reactions. These findings are consistent with those reported by (25), who demonstrated that *Moringa oleifera* leaf extracts, particularly those obtained using polar solvents such as methanol and ethanol, are rich in phenolic compounds.

Furthermore, the leaves contained 32.6 mg/g of total tannins. Tannins are high-molecular-weight phenolic compounds characterized by their ability to bind proteins and metal ions. Their presence is largely associated with the plant's natural defense mechanisms against insects and pathogenic microorganisms. In addition, tannins possess antioxidant and antimicrobial activities. However, high concentrations of tannins may reduce the bioavailability of certain nutrients through the formation of complexes with proteins and minerals (41, 33).

The total flavonoid content reached 74 mg/g,with quercetin and kaempferol like ly representing the major flavonoid compounds contributing to this value, as identified by high-performance liquid chromatography (HPLC) analysis. Flavonoids are among the most potent

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naturally occurring plant antioxidants, as they inhibit the formation of reactive oxygen species (ROS) and protect cellular membranes against oxidative damage. The relatively high flavonoid content may also be associated with the physiological stage of leaf development. Furthermore, the accumulation of these compounds is known to increase when plants are exposed to environmental stresses, such as intense light or moderate drought, reflecting their role as part of the plant's defense mechanisms against oxidative and environmental stress (34).

3.5. DPPH Radical Scavenging Activity

The results of the DPPH radical scavenging assay are presented in Table (8). As shown, the antioxidant activity of the *Moringa oleifera* leaf extract increased progressively with increasing extract concentration, indicating a positive concentration-dependent relationship between the extract concentration and its free radical scavenging capacity.

Table (8): DPPH radical scavenging activity of *Moringa oleifera* leaf extract

Extract	30 ppm	60 ppm	120 ppm	250 ppm	500 ppm
Moringa oleifera	19.05	38.98	57.44	69.08	73.65

The results of the DPPH radical scavenging assay demonstrated that the percentage of free radical inhibition increased with increasing concentrations of *Moringa oleifera* leaf extract, reaching 19.05%, 38.98%, 57.44%, 69.08%, and 73.65% at concentrations of 30, 60, 120, 250, and 500 ppm, respectively.

This gradual increase indicates that the antioxidant activity of the moringa extract is concentration-dependent, meaning that its ability to neutralize free radicals improves as the concentration of bioactive compounds in the extract increases. This activity can be attributed to the presence of phenolic compounds, flavonoids, vitamin C, and other naturally occurring antioxidants in *Moringa oleifera* leaves. These compounds donate hydrogen atoms or electrons to the DPPH free radical, resulting in a decrease in absorbance at 517 nm and, consequently, an increase in the percentage of radical scavenging activity (17).

The results also showed that the increase in radical scavenging activity became less pronounced at the higher concentrations (250 and 500 ppm), where the inhibition percentage increased only from 69.08% to 73.65%. This trend suggests that the extract was approaching its maximum antioxidant capacity, likely because the reduction of DPPH radicals became nearly saturated as the available electron- or hydrogen-donating compounds in the extract approached their maximum scavenging potential.

Overall, these findings demonstrate that *Moringa oleifera* leaf extract exhibits antioxidant activity and that its free

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radical scavenging capacity increases with increasing extract concentration.

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